Review Article

Role of the innate immunity in female reproductive tract

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Abstract

The mucosal immune system in the female reproductive tract (FRT) is well equipped to meet the sexually transmitted pathogens, allogeneic sperm, and the immunologically distinct fetus. Analysis of the FRT indicates that epithelial cells provide a physical barrier against pathogens and microbial infections as well as secretions containing anti-microbial peptides, cytokines, and chemokines which recruit and activate immune cells. Epithelial and immune cells confer protection in part through Toll-like receptors. The aim of this literature is to review the diverse components of the innate immune system, contributing to an exclusive protection system throughout the FRT.

Key Words: Anti-microbial peptides, cytokines and chemokines, female reproductive tract, immune cells, Toll-like receptors.

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INTRODUCTION

The main problem of worldwide health in reproductive field is sexually transmitted infections (STIs) and their associated diseases.[1] In spite of sustained preventive activities, only limited success has been achieved in curtailing the reproductive complexity and mortality related to STIs.[2]

Some complications of STIs with the largest prevalence and socio-economic burden include infections by Herpes simplex virus type 2, group B streptococcus, *Treponema pallidum* (syphilis), bacterial vaginosis, Hepatitis B virus (hepatitis), *Neisseria gonorrhoeae* (gonorrhea), *Chlamydia trachomatis,* and human immunodeficiency viruses (HIVs).[3] Some of them like *C. trachomatis* are associated with cervicitis, ectopic pregnancy, pelvic inflammatory disease, tubal factor infertility, spontaneous abortion, and chronic pelvic pain.[4] There are more than 20 pathogens transmissible through sexual intercourse.[3] Protection against these pathogens and others in the female reproductive tract (FRT) is provided by immune system; thus, knowing better about the immune system can help to design novel strategies which may more effectively treat STIs.

The human immune system is fundamentally divided into two major sub-divisions, the innate or non-specific and the adaptive or specific immune system.

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Although both of them have a protective function against invading pathogens, they differ in time to react, the cells involved, effector mechanisms, type, and specificity of receptors.[5,6] The innate immune system constitutes the first line of response to infection and incorporates rapidly after dealing with infectious agents, for this reason it has a pivotal role in host defense.[5-7]

The innate immune system in the FRT consists of mechanical, chemical, and cellular components. Mucus lining and epithelial cells act as the mechanical barrier. The chemical barrier can be divided into natural anti-microbial peptides (AMPs) (NAPs) and pattern recognition receptors (PRRs), specially Toll-like receptors (TLRs).[8] Briefly, NAPs are mainly produced by epithelial cells and neutrophils. They destroy target cells through abrogation of PH and ionic concentration gradients.[9] TLRs are expressed on the immune cells, including neutrophils, macrophages, dendritic cells (DCs), dermal endothelial cells, and mucosal epithelial cells.[10] They detect microbial-associated molecular patterns and gather a number of adapters to initiate intra-cellular signaling pathways in order to recruit the immune cells, to secret anti-microbial factors eradicating pathogens, and finally, to facilitate adaptive immune responses.[1,6,8,11]

The cellular components include inflammatory immune cells that migrate into the genital tract, as well as resident epithelial cells and stromal fibroblast.[8]

The mucosal innate immune system of the FRT is not only involved in specialized physiological events, including menstruation, fertilization, implantation, pregnancy, and parturition, but also in protecting against sexually transmitted pathogens, as well as supporting allogeneic spermatozoa and an immunologically distinct fetus. To meet these challenges, the FRT has unique requirements as mentioned above, briefly.[6] The purpose of this review article is to examine key mediators of innate immune defense that protect female genital tract against pathogens.

EPITHELIAL CELLS BARRIER, MUCUS

The FRT consists of three compartments: Lower part (vagina and ectocervix), transitional endocervix, and the upper part (endometrium and the fallopian tubes). All of them are covered by epithelial cells which, on one hand, provide a physical and immunological barrier to protect against invading micro-organisms, on the other hand, support the migration of sperm, ovum, and fetus.[12] Integrity of the mucosal monolayer in the upper FRT is preserved with tight junctions between columnar epithelial cells. However, the lower part is lined with multiple layers of stratified squamous epithelial cells, containing a loose connection.[13] Entirety of mucosal epithelial barrier can be directly altered by sex hormones, cytokines, growth factors, TLR agonists, and pathogens.[12,14,15] A lack of tight junctions in the lower part of the FRT may permit transition of intruder to intra-epithelial, which results in pathogens' counter with immune cells like $CD4^{+}T$.[16] On the other hand, PRRs, which are located on epithelial cells, detect antigens on these micro-organisms and then induce secretion of AMPs, cytokines, and chemokines. Totally, mucosal epithelial cells play important roles in innate immunity by: (I) formation of a physical and immunological barrier, (II) sending signals to the underlying immune system, (III) production of cytokine and chemokine, (IV) inducing death of infected cell through necrosis, apoptosis, or phagocytosis, (V) activating adaptive immunity, and (VI) development of an acute inflammatory reaction.[17]

The epithelial cells of endometrium as well as vagina are covered by a layer of mucus, which maintains them from direct contact with infectious agents.[13]

Most of the components of mucus are water and a family of high-molecular-weight glycoproteins, namely mucin, particularly mucin-1, which traps micro-organisms.[18] Domino *et al*. showed in an animal model that cervical mucins have a protective role against *Candida albicans*.[19] Human cervico-vaginal mucus provides a protective barrier blocking the spread of STIs from the vagina toward the upper FRT.[13] Poor secretion of cervico-uterine mucus seems probably to be related to reduced fertility in women with cystic fibrosis.[20] The properties and amount of the secreted mucus vary during the menstrual cycle under the influence of the sex hormones. Estrogenic mucus is present at the proliferative stage and increases at mid-cycle. It is less viscous and appears to provide a more favorable environment for sperm migration.[21] Progestational mucus is present at high level following ovulation and low level during the menstrual and early proliferative phases. It is thick, sticky, and restricts the passage of sperm into the uterus.[21] It can be concluded that epithelial cells and mucus are two key components of the physiological barrier which protects the FRT against pathogens.

TOLL-LIKE RECEPTORS

Rapid innate immune defense against infection usually involves the detection of pathogen-associated molecular patterns (PAMPs) and endogenous damage-associated molecular patterns (DAMPs) by specific PRRs.[10,22,23]

Recent studies identified several classes of PRRs family including TLRs, nucleotide oligomerization domain (NOD)-like receptors (NLRs), retinoic acid-inducible gene I (RIG-I)-like receptors (RLRs) and cytosolic DNA sensors.[24] Toll protein was discovered for its role in dorsoventral patterning of *Drosophila* embryos. Later investigations declared an important role of Toll in the fly's immune response to bacterial and fungal infections that have opened a new window for the mammalian homologues research.[11,25] The first identified human TLR was TLR4.[26] Structurally, the TLRs are comprised of an extra-cellular leucine-rich repeat domain that recognizes PAMPs and a cytoplasmic Toll/interleukin 1 receptor domain for downstream signaling transduction.[27,28] TLRs have a critical role in the induction of immune and inflammatory responses in mammals.[23] To date, at least 10 human TLRs and 13 mouse TLRs have been described[11] TLR1, 2, 4, 5, and 6 are located on the plasma membrane and detect pathogen membrane components, while TLR3, 7, 8, and 9 are expressed in cytoplasmic organelles, mainly the endosomes, lysosomes, endolysosomes, and endoplasmic reticulum in order to detect pathogen nucleic acids.[11,29] TLR1-9 are conserved between human and mice. Mouse TLR 11 is functional, but the human homolog has a stop codon that results in the lack of production.[23] Each individual TLR has a distinct function in terms of PAMPs detection and immune responses.[30] Examples of PAMPs include lipopolysaccharide (LPS), the major component of gram-negative bacterial outer membranes, peptidoglycan, and the major component of gram-positive bacterial cell walls, lipoproteins, zymosan, and nucleic acids.[31] On the other hand, heat-shock protein 60 and 70, polysaccharide fragments of heparin sulfate, hyaluronic acid, fibrinogen, fibronectin DA domain, and mRNA are also categorized as DAMPs.[8] Table 1 shows an overview of the cognate ligands for TLRs.[29,30,32-40]

TLRs do not act alone. Their signal transduction is mediated by the recruitment of different intra-cellular adaptors [Table 1].[11] Selective usage of these adaptor molecules causes differential responses mediated by these distinguished distinct TLR ligands.[29] Two intra-cellular signaling cascades can be induced after TLR activation, MyD88-dependent cascades, which lead to secretion of pro-inflammatory cytokines or TRIF-dependent cascades, which induce type 1 interferon (IFN) as well as inflammatory cytokines and chemokines [Figure 1].[30] After the discovery of TLRs, other PRRs, comprising of NLRs such as RLRs, were identified. Similar to TLRs, NLRs and RLRs have an important role in immune responses; however, in contrast to TLRs, they only detect microbial components in the cytosol. NLR family has more than 20 members and is involved in response to the various PAMPs and PAMP particles through production of IL-1β.[24,30]

TLRs in the female reproductive tract

The mucosal epithelium of the genital tract serves as front line of defense against microbial infections. It has been thought the expression of TLRs on the epithelium plays an important role in antigen detection, initiation of immune response, and connection between innate and adaptive immunity.[8] Recent studies have also supported the importance of TLRs activation in fertilization and implantation failure through stimulation of the innate immune system.[41,42] Several research works have been done on the expression and role of these receptors in the FRT.[2,43,44]

Constitutive expression of TLR1-10 in epithelial cells of fallopian tubes and endometrium has been reported.[2,43,45,46] The presence of TLR1-9 has also been detected in vagina and cervix.[43,47-49] TLR1-3, 6, 7, and 10 exist in uterine natural killer (NK) cells[50,51] and TLR1 is present in vascular endothelial and smooth muscle cells of the cervix and uterus.[10] Expression of TLR2-4, 7-10 has been shown in endometrial stroma.[52,53] Other reports have demonstrated the existence of TLR5 in smooth muscle and vascular endothelial cells within the stroma of the vagina and endocervix [Figure 2].[10]

The presence of TLR2 and TLR4 on amniotic epithelial cells has also been shown during pregnancy; however,

Table 1: Overview of used adaptor proteins by TLRs.

Adaptor protein	Ligand	Receptor number
MyD88/Mal	Triacyl lipopeptides, Pam_3Cys-Ser-$(Lys)_4$	TLR1
MyD88/Mal	peptidoglycan, lipoprotein, Pam_3Cys-Ser-$(Lys)_4$, Zymozan, and lipoteichoic acid	TLR2
TRIF	dsRNA (virus), siRNA, endogenous mRNA, and poly (I:C)	TLR3
TRIF/MyD88/Mal/TRAM	LPS, lipid A analogs, cryptococcal capsule, Aspergillus hyphae, respiratory syncytial virus Protein F, heat shock protein 60, 70 and fibronectin, and hyaluronic acid. LPS derived from *N. gonorrhoeae*, LPS and HSP derived from *C. trachomatis* and mannan derived from *C. albicans*	TLR4
MyD88	Flagellin	TLR5
MyD88/Mal	diacyl lipopeptide, soluble tuberculosis factor	TLR6
MyD88	ssRNA, imiquimod, resiquimod and loxoribine (anti-viral and anti-tumoral compounds)	TLR7
MyD88	ssRNA	TLR8
MyD88	Unmethylated CpG DNA, ssRNA	TLR9
MyD88	bacterial lipopeptide ligands	TLR10

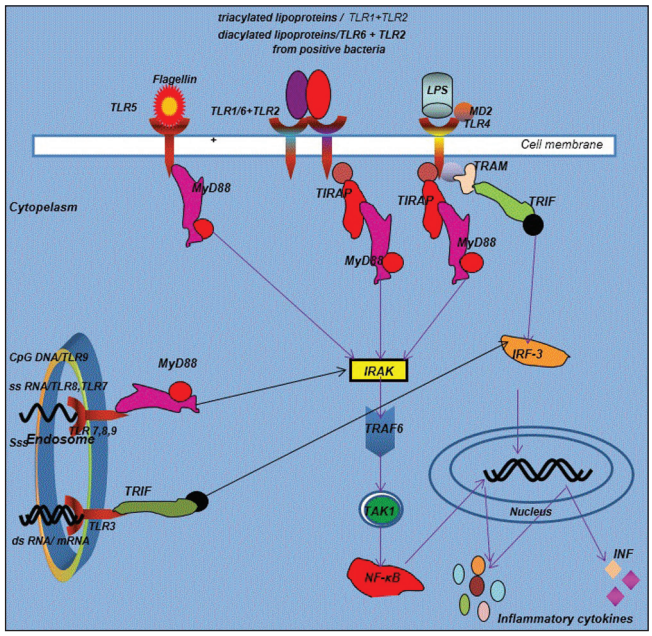

Figure 1: TLRs signaling pathway

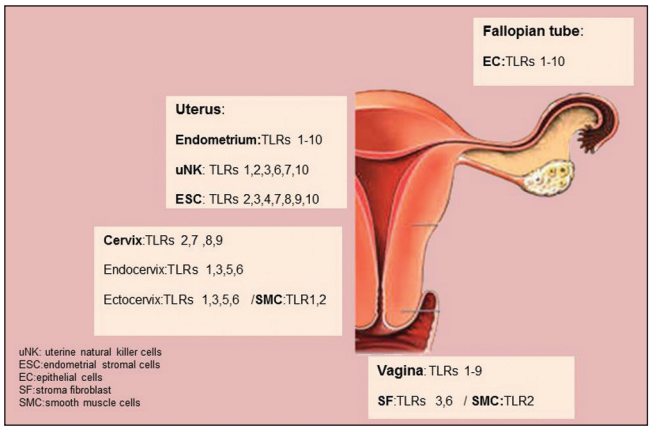

Figure 2: Localization of TLRs in the female reproductive tract

the presence of TLR2 seems to be restricted to the basolateral side of these cells.[54] TLR2 functions as a heterodimer with either TLR1 or TLR6 on the plasma membrane of both innate and adaptive immune cells. The TLR2/TLR1 heterodimers detect triacylated lipoproteins from gram-negative bacteria and mycoplasma, whereas TLR2/TLR6 heterodimers recognize diacylated lipoproteins from gram-positive bacteria and mycoplasma.[30]

TLR4 proteins have been detected in term decidual inflammatory immune cells, such as neutrophils and macrophages.[23]

The expression of TLR4 decreases from the upper genital tract toward cervix.

There is controversy about the presence of TLR4 in epithelial cells of the female genital tract. Some reports have declared the presence of TLR4 in the epithelial cells of the fallopian tubes, endometrium, endocervix, and vagina, while others have rejected this report.[10,43,47,55,56]

TLR4 is involved in the response to LPS of gram-negative bacteria in association with CD14 and MD-2.[11,57] CD14 is found in endometrial stromal fibroblasts, but not in endometrial and fallopian epithelial cells.[56,58] Unlike epithelial cells in the upper part of FRT, epithelial cells of cervix and vagina express co-receptor CD14 (55). It was reported that MD2, an ancillary molecule of TLR4-signaling, was missing in cultured epithelial cells derived from normal human vagina, ectocervix, and endocervix.[8,47] Recently, Packiam *et al*. have experimentally demonstrated that TLR4 has a protective role against gonococcal infection.[59]

TLR3 activates by double-stranded RNA and mRNA from killed cells.[60] It has been shown that TLR3 function can depend on sexual hormones. For example 17-β-estradiol inhibits cytokine and chemokine production which are already induced by TLR3 activation without any effect on TLR3 expression.[61]

TLR9 recognizes CpG motifs in the genome of bacterial and viral pathogens. Production of IL-8 by cultured epithelial cells of fallopian tubes, uterine, and cervix increases in response to binding CpG oligonucleotides to TLR9.[49]

Aflatoonian *et al*. have demonstrated the cycle-dependent expression of TLR1-10 in human endometrium. They declared that relative expressions of TLR2-6, 9, and 10 were significantly higher during the secretory phase compared to other phases of the menstrual cycle. According to these findings, we can probably conclude the inhibitory effect of estrogen or the protective effect of progesterone in the genital tract, especially in the endometrium.[2,62]

NATURAL ANTI-MICROBIAL PEPTIDES

Synthesis of peptides and small proteins with anti-microbial activity is generally emerged as the most ancient primary mechanism of the immune system.[9] NAPs possess additional functions apart from microbicidal activity, including cell proliferation, cytokine induction, chemotaxis, and modulation of innate and acquired immunity.[63]

Endogenous AMPs have redundancy and synergism together, so these properties provide better protection in comparison to a single factor.[64] Major NAPs with different structural and functional characteristics

include defensin, elafin, cathelicidin, secretory leukocyte protease inhibitor (SLPI), lysozyme, and lactoferrin. They are mainly produced by epithelial cells and neutrophils[9] and regulated by bacterial production and inflammation.[65] It has shown that AMPs similar to antibodies, cytokines, and chemokines vary at different times during the menstrual cycle reflecting endocrine regulation. Also, it is noticeable that biological activity of antimicrobials can change with pH, salt, serum, and presence of sperm.[3,66,67] NAPs can interact with cell membrane of pathogens based on the charge, then forming pores that destroy target cell through abrogation of pH and ionic concentration gradients.[9] Together, NAPs constitute an important chemical barrier which orchestrates immune responses against foreign micro-organisms.

Defensins

One of the most prominent NAPs at the mucosal surface is defensins. Two main functional sub-families of them are α and β-defensins. Six α-defensins have been recognized in humans: (I) HNP (human neutrophil peptide) 1-4 and (II) HD5,6 (human defensin). Leukocytes and epithelial cells are the main sources of HDs.[1] α-Defensins have anti-bacterial activity against gram-negative and gram-positive bacteria, fungi, yeast, and anti-viral effects against HIV-1, 2, and HSV-1; however, α-defensins 5 and 6 increase HIV infection.[3] Six human β-defensins, HBD1 to 6 have been identified, which are structurally similar to α-defensins. Four of them are expressed by mucosa and epithelial cells of the female genital tract.[3,68,69] They have anti-viral activity and decrease level of HIV-1CXCR4 co-receptor.[70,71] Several studies have examined the presence and role of defensins in the FRT at different stages of menstrual cycle.[1,72,73] It has been shown that HBDs1-4 and α-defensing 5 are expressed in the endometrial epithelium. HBDs1, 3, and 5 are at maximal concentration during the secretory phase while HBD4 reaches peak in the proliferative phase and HBD2 is highest during the menstruation.[1,73] Within the cervico-vaginal lavages (CVL), HNPs1-3 and HBD2 are maximum during the proliferative phase and minimum at mid-cycle.[74]

During pregnancy, endogenous anti-microbials can play a critical role in preservation of uterine health and prevention of its infection. Expression of α- and β-defensins has been detected in the amnion epithelium, chorion, decidua, trophoblast, and cervical mucus plug, during pregnancy. In addition, changes in vaginal microflora are related to defensins at mid-pregnancy.[75]

Elafin and secretory leukocyte protease inhibitor

SLPI and elafin from whey acidic protein family were introduced as human protease inhibitors.[76] SLPI is synthesized by macrophages and epithelial cells. It suppresses elastase and cathepsin G, but not proteinase3, while elafin is inhibitor for elastase and proteinase3. The anti-protease effect of these peptides can restrict host tissue damage from an unregulated inflammation, in part mediated by proteases.[9]

SLPI has anti-bacterial activity (against Escherichia coli, *Pseudomonas aeruginosa*, *Staphylococcus aureus*) and anti-fungal activity (against *Aspergillus fumigatus* and *C. albicans*.[77] Elafin and SLPI also have anti-HIV activity in vaginal fluid that is independent of their protease inhibitory role.[78,79] SLPI and elafin are present throughout FRT.[1] SLPI expression has been detected in the vagina, cervix, amnion, vernix caseosa, uterus pregnant, and decidua and at very high level in cervical mucus (1000 μg/mL).[80,81] Elaphin expresses in the vagina, cervix, uterus pregnant, fetal membranes, and placenta just at term pregnancy.[3] Endometrial neutrophils are rich source of Elafin during menstruation. SLPI and elafin are expressed during pregnancy probably for anti-inflammatory, anti-protease, and anti-microbial properties.[1]

Cathelicidin LL37, lactoferrin, and lysozyme

Another component of FRT secretions is cathelicidin. In humans, LL37 is only cathelicidin, which is produced by neutrophils and epithelial cells of the lower FRT.[3,9] LL37 is found in vaginal fluid and cervical mucus. It counters with bacteria and fungi which may have been introduced by intercourse.[81]

Main sources of lactoferrin are neutrophils and epithelial secretions. It has been found in vaginal fluid (1 μg/mL) and cervical mucus (100 μg/mL).[82] It has anti-viral and anti-bacterial effects (against gram-negative bacteria), directly or by sequestration of iron essential for microbes under acidic conditions, such as lower part of FRT.[81-83] Lactoferrin displays synergism with lysozyme that promotes innate immune protection in the FRT.[7]

Lysozyme is synthesized by neutrophils and detected in vaginal fluid (13 μg/mL) and mucus plug (1 mg/mL).[82] In addition to enzymatic lysis of peptidoglycan present on bacterial cell walls, lysozyme can kill bacteria by a non-enzymatic mechanism. Although lysozyme has an anti-bacterial effect against gram-positive species, for example streptococci, but it is in effective against gram-negative bacteria.[84] It also blocks HIV-1 viral entry and its replication.[85,86]

CYTOKINES AND CHEMOKINES

Cytokines are small pleiotropic glycoprotein mediators whose biological actions are locally mediated

by specific receptors.[75] Chemokines are small chemotactic cytokines, very locally acting, well known for their function in leukocyte recruitment to sites of inflammation and their activation.[87] Chemokines attract immune cells to the tissue, while cytokines differentiate and activate these cells.[6] Several studies have demonstrated the constitutive secretion of numerous cytokines, including granulocyte-macrophage colony-stimulating factor (GM-CSF), granulocyte colony-stimulating factor (G-CSF), TNF-α, IL-1, IL-6, leukemia inhibitory factor (LIF), TGF-β, and of chemokines such as MIP-1β, monocyte chemoattractant protein-1 (MCP-1), and IL-8 by polarized epithelial cell from the cervix, uterus, and fallopian tubes.[12,88-91] Most of these inflammatory mediators were preferentially secreted into the apical/luminal compartment resulting in a gradient for stimulation and attracting immune cells to the epithelial surface.[12,92] For example, IL-8 produced by uterine epithelial cells induces neutrophil migration across the epithelium.[92,93] Also, MCP-1 and MIP-1β are potent chemoattractants for monocytes and T cells, respectively. Other cytokines, such as TGFβ, secreted into the baso-lateral/sub-epithelial compartment of uterus, affect function and development of immune cells.[94,95] The cytokines TNFα, IL-6, GM-CSF, and G-CSF trigger differentiation of leukocytes to more active pro-inflammatory cells.[12] In addition to chemotactic activity, IL-8 participates in proliferation and angiogenesis during early to mid-secretory phase, as well as in apoptosis during menstruation.[91] The type I IFNs are other important cytokine family involved in FRT immunity, particularly against viruses.[96] IFNs are immediately induced in counter with viral and bacterial pathogens.[60,97] IFNβ is induced in uterine epithelial cells by the double-stranded viral agonist poly (I:C). In the FRT, IFNβ induces expression of the anti-HIV molecules, like MIP3α/CCL20 and hBD2, showing its protective role against HIV-1 infection.[13] Immune cells, including monocytes, macrophages, NK cells, and DCs, are also the sources of immunoregulatory cytokines and chemokines in the FRT.[98,99] The secretion of chemokines and cytokines is modulated by autocrine and paracrine manners, as well as sex hormones. The hormonal effect on their secretion is direct or indirect.[12,13] For instance, progesterone depletion leads to the up-regulation of IL-8, MCP-1, and COX-2, resulting in the activation of monocytes and neutrophils and finally, up-regulation of matrix metalloproteinases for initiation of menstruation.[100] As an indirect effect, estradiol treatment leads to up-regulation of hepatocyte growth factor (HGF) secretion that in turn regulates TNFα and MIP3α/CCL20 production by uterine epithelial cells.[101-103] Thus, concentrations of chemokines and cytokines will vary in the endometrium during normal physiological processes, as well as pathological conditions, such as infection and endometriosis.[92] Production of chemokines and cytokines by uterine epithelial cells may associate with pathological conditions during pregnancy. For example, there is a relationship between elevated concentrations of IL-6, IL-8, and MCP-1 and amniotic microbial infection in cervical and amniotic fluids from patients with spontaneous preterm birth.[104-106] In contrast, low levels of IL-1β, IL-8, and IL-6 in cervical fluid are correlated with clinical chorioamnionitis in early pregnancy.[107] So, low concentrations of cytokines create a permissive environment for ascending infection. It has been recommended that the levels of specific chemokine(s) and cytokine(s) in the FRT during pregnancy should be monitored where is a high risk of preterm labor.[108] Expressions of cytokines, chemokines, and adhesion molecule are also critical for endometrial growth in preparation for fertilization, implantation, and successful pregnancy, but also for the remodeling of the uterus during each menstrual cycle that is regulated by the sex hormones.[109-111]

Collectively, cytokines and chemokines, as chemical messengers, provide an immunological environment hostile to pathogen survival and maintain the normal homeostatic.[91] Their secretion leads to rapid communication between the different immune cells which are present in the FRT.[13] Innate immune cells provide another line of defense.

INNATE IMMUNE CELLS

Macrophage

As professional phagocytes, macrophages play an important role in the pathogens recognition, removal of debris, and indirect stimulation of the immune system by cytokines and chemokines production along with all aspects of inflammatory responses. These versatile cells are widely distributed throughout the human FRT (constitute 10% of the leukocytes population in the FRT).[6,112] The numbers of endometrial macrophages increase prior to menstruation, and also macrophage chemoattractants, like MCP-1, FKN, and MIP-1β, are up-regulated peri-menstrually.[6,113] Accumulation of endometrial macrophages also occurs across the mid-secretory phase of the cycle, while numbers of vaginal macrophages remain constant during the menstrual cycle.[13] Increasing evidences suggest that the migration of macrophages into endometrium is modulated by estradiol and progesterone.[114-116] Physiologic levels of estrogen induce macrophage proliferation and function.[117] Tissue macrophages have different phenotypic characteristics, reflecting the unique local micro-environment which they have been exposed.[6] For example, vaginal macrophages

express higher levels of the HIV-1 receptor CD4, CCRs, and CXCR4.[118,119] Macrophages have also been identified as key modulators of ovarian function through regulation of folliculogenesis and atresia.[120] They are most numerous during ovulation as an inflammatory reaction.[117] Decidual macrophages can participate in diverse activities during pregnancy.[121] They are classified as M1- and M2-types which take part, respectively, in progression of inflammation and immune tolerance during pregnancy. A balance of them may contribute to the outcome of pregnancy.[121]

Dendritic cells

DCs, as the major antigen-presenting cells in the FRT, seem to make a link between innate and adaptive immunity. They are present in the sub-epithelial stroma of the endometrium. In contrast, vaginal DCs are localized to the epithelial layer.[122] Exposure to pathogens and inflammatory stimuli, such as LPS, lead to maturation of DCs which are characterized by CD38 marker expression and IL12 production. Mature DCs facilitate the development of T-helper 1 (Th1) cells.[122] The roles of DCs are to prevent infection by direct inactivation or the stimulation of adaptive immunity. However, new findings have suggested the expression of DC-SIGN by these cells could increase susceptibility of women to HIV infections.[123] DCs are not only essential for the induction of primary immune responses, but are also important for the induction of immunological tolerance and maintenance of successful pregnancy.[124,125] They are recruited into the endometrium and accumulated especially around the implanted embryo.[124] The function and differentiation of DCs are regulated by the local microenvironment determined by cytokines, chemokines, and estroied hormones.[124] Estradiol has been shown preferentially through a promotion of a specific sub-set of DCs differentiation, characterized by high surface expression of MHC class II and CD86.[126]

Natural killer cells

NK cells, as the key innate immune cells, use a variety of effector mechanisms to promote host immune defenses, while eliminating virus-infected cells and tumor cells by secretion of cytotoxic products.[127] Defects in NK cell activity are associated with increased infections particularly, herpes viral infections, ovarian cancer, uterine cancer, and endometriosis,[6,128-131] whereas elevated NK-cell activity has been associated with recurrent pregnancy loss.[132,133] NK cells have the ability to amplify an inflammatory response and to promote macrophage activation, generation of cytotoxic T cells, recognition of fungal infections, and cytokine production.[6,134,135] Uterine NK cells (uNKs) express several TLRs in particular TLR2, TLR3, and TLR4 which can respond to TLR agonists by producing cytokines.[123] Variety of cytokines, including IL12, IL8, IL15, IL1β, or IFNα in combination with PAMPs will activate NK cell cytokine production, leading to further activation of innate immunity.[136-138] The number of endometrial NK cells are low in the early proliferative phase and increase as the menstrual cycle progresses, reaching a peak in the late secretory phase.[112,139,140] However, NK cell numbers in other regions of the FRT are not affected across the menstrual cycle.[13] Also, an increase in number of endometrial NK cells increase during early pregnancy reaches a maximum at the end of the first trimester and a minimum at term.[141] It shows an important role of these cells in the establishment and maintenance of pregnancy. At least, two theories have been proposed for the increase of uNK cells within the uterus: *In situ* proliferation and recruitment from the peripheral NK cells blood.[141-144] Estradiol regulates NK cells activity *via* endogenous TGFβ.[123] Several reports suggest that IL15 is also required for uNK cells survival, proliferation, and differentiation into decidual NK cells.[144-149] The uNK cells have a distinct phenotype from blood NK cells.[150] Unlike blood NK cells, uNk cells express CD9 and CD69 on their cell surface.[99,151-153] It has been shown the cell-surface phenotype of NK cells is different within the FRT. For example, CD69 and CD96 are both expressed by NK cells in the endocervix and endometrium, but not in the ectocervix.[150] Uterine NK cells, not blood NK cells produce some essential cytokines for implantation such as, angiogenic growth factors (vascular endothelial growth factor (VEGF), Placental growth factor(PLGF) , Angiopoietin2) and leukemia inhibitory factor (LIF).[13,154,155] Finally, it can be concluded that uNK cells are involved in several processes, including host defense, decidualization, implantation, and pregnancy.[141-143]

Neutrophil

Neutrophils are present in all tissues of the FRT and possess many effector mechanisms for mediating innate immunity.[156] Under the influence of chemokines gradient,[157,158] neutrophils can cross the endothelial barrier, eliminate pathogens by phagocytosis, and produce toxic oxygen and nitrogen species, as well as release cytokines and anti-microbial compounds, such as defensin–serine proteases.[159-161] IL8 is a major neutrophil chemoattractant.[162] IL8 and GM-CSF, secreted by epithelial cells, cause to bring neutrophils toward the epithelium or cross the epithelial barrier into the lumen.[6] Insemination also causes a great influx of neutrophils into the uterine lumen to remove superfluous sperm, micro-organisms, and seminal debris. This migration is accompanied by accumulation of macrophages, DCs, granulocytes, and lymphocytes in the endometrial stroma to maintain uterine sterility.[163] In contrast

to NK cells, neutrophil numbers are highest in the fallopian tubes whereas progressively decrease from the upper FRT into the lower regions of the tract.[112] In spite of the most numerous neutrophil in the fallopian tubes, their exact role remains to be studied.[164] The number of neutrophils in vagina has been shown to be stable throughout the cycle, similar to T cells and macrophages, expect in vaginal fluid from infected women.[165,166] Also, it has been declared that neutrophils count does not fluctuate across the menstrual cycle, but at menses sharply increase in the endometrium, which is preceded by a surge in IL-8.[13] At menses, breakdown of endometrial tissue is done by neutrophils *via* the release of elastase which activates matrix-metalloproteinases.[6,13] Some evidence suggest that exposure to different cytokines within FRT tissue can arise a different neutrophil population by altering their function and receptor expression.[167] For example, fallopian tube neutrophils express higher level of CD15 marker which may be important in innate immune defense of the fallopian tube.[168]

Our knowledge regarding immune defense mechanisms in the FRT remains limited. By further studies, new avenues may be identified both to protect against pathogens and to improve the quality of woman's reproductive health.

CONCLUSIONS AND FUTURE PERSPECTIVES

Growing body of data about the FRT demonstrates the presence of a complex system of immune protection. Mucus lining, a tight epithelial barrier, the secretion of AMPs and cytokines by epithelial and innate immune cells, and expression of TLRs throughout the reproductive tract indicate that the FRT has evolved to meet the challenges of STIs and to minimize the risk of infection in order to support an allogeneic fetus. This review confers the opportunity of understanding the unique immunological characteristics of the female genital tract, and also highlights the need for further researches. Finally, we hope to provide new approaches into design novel therapeutic means for the female reproductive diseases associated with the innate immune system.

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